09/040103

Search result for Page, #22

DB Name	Query	Hit Count	Set Name
USPT,PGPB,JPAB,EPAB,DWPI,TDBD	l7 and Moloney near10 probe$	1	L12
USPT,PGPB,JPAB,EPAB,DWPI,TDBD	"Mv-1-Lu" and galactosyl	0	L11
USPT,PGPB,JPAB,EPAB,DWPI,TDBD	"Mv-1-Lu" near10 galactosyl	0	L10
USPT,PGPB,JPAB,EPAB,DWPI,TDBD	l7 and Moloney near10 hybridiz$	1	L9
USPT,PGPB,JPAB,EPAB,DWPI,TDBD	l7 and Moloney	23	L8
USPT,PGPB,JPAB,EPAB,DWPI,TDBD	l6 and serum near5 resist$	48	L7
USPT,PGPB,JPAB,EPAB,DWPI,TDBD	retrovir$ near5 vector$	5469	L6
USPT,PGPB,JPAB,EPAB,DWPI,TDBD	MDOK and retrovir$ near5 vector$	0	L5
USPT,PGPB,JPAB,EPAB,DWPI,TDBD	"Mv-1-Lu" and MDOK and retrovir$ near5 vector$	0	L4
USPT,PGPB,JPAB,EPAB,DWPI,TDBD	"Mv-1-Lu" and retrovir$ near5 vector$	11	L3
USPT,PGPB,JPAB,EPAB,DWPI,TDBD	MPF and retrovir$ near5 vector$	12	L2
USPT,PGPB,JPAB,EPAB,DWPI,TDBD	MPF and retrovir$ vector$	193000	L1

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Terms	Documents
l7 and Moloney near10 probe$	1

Database:
- US Patents Full-Text Database
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☐ 1. Document ID: US 6168916 B1

L8: Entry 1 of 23 File: USPT Jan 2, 2001

US-PAT-NO: 6168916
DOCUMENT-IDENTIFIER: US 6168916 B1

TITLE: Host adaptation of retroviral vectors

DATE-ISSUED: January 2, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kingsman; Alan John	Oxon	N/A	N/A	GBX
Kingsman; Susan Mary	Oxon	N/A	N/A	GBX
Cannon; Paula Marie	South Pasadena	CA	N/A	N/A
Nowak; Martin Andreas	Oxford	N/A	N/A	GBX

US-CL-CURRENT: 435/5; 435/235.1, 435/236, 435/237, 435/320.1, 435/325, 435/455, 435/456, 435/6, 435/7.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 2. Document ID: US 6165715 A

L8: Entry 2 of 23 File: USPT Dec 26, 2000

US-PAT-NO: 6165715
DOCUMENT-IDENTIFIER: US 6165715 A

TITLE: Expression systems

DATE-ISSUED: December 26, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Collins; Mary Katherine Levinge	London	N/A	N/A	GBX
Weiss; Robin Anthony	London	N/A	N/A	GBX
Takeuchi; Yasuhiro	London	N/A	N/A	GBX
Cosset; Francois-Lois	Lyons	N/A	N/A	FRX

US-CL-CURRENT: 435/6; 435/320.1, 435/325, 435/354, 435/366, 435/371, 435/372.1, 435/455, 435/456, 435/69.1, 536/23.1, 536/24.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

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☐ 3. Document ID: US 6150138 A

L8: Entry 3 of 23 File: USPT Nov 21, 2000

US-PAT-NO: 6150138
DOCUMENT-IDENTIFIER: US 6150138 A

TITLE: Expression of a foamy virus envelope protein

DATE-ISSUED: November 21, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lindemann; Dirk	Rimpar	N/A	N/A	DEX
Rethwilm; Axel	Wurzburg	N/A	N/A	DEX

US-CL-CURRENT: 435/69.7; 435/325, 536/23.4

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

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☐ 4. Document ID: US 6111087 A

L8: Entry 4 of 23 File: USPT Aug 29, 2000

US-PAT-NO: 6111087
DOCUMENT-IDENTIFIER: US 6111087 A

TITLE: Expression of a foamy virus envelope protein

DATE-ISSUED: August 29, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Rethwilm; Axel	Wurzburg	N/A	N/A	DEX
Lindemann; Dirk	Rimpar	N/A	N/A	DEX
Winter; Arend Jan	Strasbourg	N/A	N/A	FRX

US-CL-CURRENT: 536/23.4; 424/207.1, 435/69.7, 530/388.35

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

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☐ 5. Document ID: US 6093539 A

L8: Entry 5 of 23 File: USPT Jul 25, 2000

US-PAT-NO: 6093539
DOCUMENT-IDENTIFIER: US 6093539 A

TITLE: DNA encoding the T cell surface protein T4 and use of fragments of T4 in the treatment of AIDS

DATE-ISSUED: July 25, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Maddon; Paul J.	New York	NY	N/A	N/A
Littman; Dan R.	San Francisco	CA	N/A	N/A
Chess; Leonard	Scarsdale	NY	N/A	N/A
Axel; Richard	New York	NY	N/A	N/A
Weiss; Robin	London	N/A	N/A	GBX
McDougal; J. Steven	Atlanta	GA	N/A	N/A

US-CL-CURRENT: 435/6; 435/252.3, 435/254.2, 435/320.1, 435/325, 435/348, 435/69.1, 536/23.5, 536/24.31

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

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☐ 6. Document ID: US 6025127 A

L8: Entry 6 of 23 File: USPT Feb 15, 2000

US-PAT-NO: 6025127
DOCUMENT-IDENTIFIER: US 6025127 A

TITLE: Nucleic acid mutation detection in histologic tissue

DATE-ISSUED: February 15, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Sidransky; David	Baltimore	MD	N/A	N/A

US-CL-CURRENT: 435/6; 536/23.1, 536/23.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

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☐ 7. Document ID: US 6017761 A

L8: Entry 7 of 23 File: USPT Jan 25, 2000

US-PAT-NO: 6017761
DOCUMENT-IDENTIFIER: US 6017761 A

TITLE: Method for obtaining retroviral packaging cell lines producing high transducing efficiency retroviral supernatant

DATE-ISSUED: January 25, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Rigg; Richard J.	Mountain View	CA	N/A	N/A
Chen; Jingyi	Fremont	CA	N/A	N/A
Dando; Jonathan S.	Milan	N/A	N/A	ITX
Plavec; Ivan	Menlo Park	CA	N/A	N/A
Forestell; Sean P.	Menlo Park	CA	N/A	N/A
Bohnlein; Ernst	Los Altos	CA	N/A	N/A

US-CL-CURRENT: 435/455; 435/320.1, 435/325, 435/350, 435/357, 435/363, 435/364, 435/366, 435/369, 435/371, 536/23.72

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

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☐ 8. Document ID: US 5985847 A

L8: Entry 8 of 23 File: USPT Nov 16, 1999

US-PAT-NO: 5985847
DOCUMENT-IDENTIFIER: US 5985847 A

TITLE: Devices for administration of naked polynucleotides which encode biologically active peptides

DATE-ISSUED: November 16, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Carson; Dennis A.	Del Mar	CA	N/A	N/A
Raz; Eyal	San Diego	CA	N/A	N/A

US-CL-CURRENT: 514/44; 424/278.1, 424/94.1, 435/285.1, 604/46

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw. Desc | Image

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☐ 9. Document ID: US 5958678 A

L8: Entry 9 of 23 File: USPT Sep 28, 1999

US-PAT-NO: 5958678
DOCUMENT-IDENTIFIER: US 5958678 A

TITLE: DNA encoding the T cell surface protein T4 and use of fragments of T4 in the treatment of AIDS

DATE-ISSUED: September 28, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Maddon; Paul J.	New York	NY	N/A	N/A
Littman; Dan R.	San Francisco	CA	N/A	N/A
Chess; Leonard	Scarsdale	NY	N/A	N/A
Axel; Richard	New York	NY	N/A	N/A
Weiss; Robin	Finchley	N/A	N/A	GBX
McDougal; J. Steven	Atlanta	GA	N/A	N/A

US-CL-CURRENT: 435/6; 424/184.1, 424/85.1, 530/350

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw. Desc | Image

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☐ 10. Document ID: US 5952225 A

L8: Entry 10 of 23 File: USPT Sep 14, 1999

US-PAT-NO: 5952225
DOCUMENT-IDENTIFIER: US 5952225 A

TITLE: Retroviral vectors produced by producer cell lines resistant to lysis by human serum

DATE-ISSUED: September 14, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Pensiero; Michael	Dickerson	MD	N/A	N/A
Collins; Mary K. L.	London	N/A	N/A	GBX
Cosset; Francois-Loic	London	N/A	N/A	GBX
Takeuchi; Yasuhiro	London	N/A	N/A	GBX
Weiss; Robin A.	London	N/A	N/A	GBX

US-CL-CURRENT: 435/352; 435/320.1, 435/325, 435/366, 435/369, 435/371

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw. Desc | Image

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☐ 11. Document ID: US 5929222 A

L8: Entry 11 of 23 File: USPT Jul 27, 1999

US-PAT-NO: 5929222
DOCUMENT-IDENTIFIER: US 5929222 A

TITLE: Expression of a foamy virus envelope protein

DATE-ISSUED: July 27, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lindemann; Dirk	Rimpar	N/A	N/A	DEX
Rethwilm; Axel	Wurzburg	N/A	N/A	DEX

US-CL-CURRENT: 536/23.4; 435/69.7

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw. Desc | Image

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☐ 12. Document ID: US 5910434 A

L8: Entry 12 of 23 File: USPT Jun 8, 1999

US-PAT-NO: 5910434
DOCUMENT-IDENTIFIER: US 5910434 A

TITLE: Method for obtaining retroviral packaging cell lines producing high transducing efficiency retroviral supernatant

DATE-ISSUED: June 8, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Rigg; Richard J.	Mountain View	CA	N/A	N/A
Chen; Jingyi	Fremont	CA	N/A	N/A
Dando; Jonathan S.	Sunnyvale	CA	N/A	N/A
Plavec; Ivan	Menlo Park	CA	N/A	N/A
Forestell; Sean P.	Menlo Park	CA	N/A	N/A
Bohnlein; Ernst	Los Altos	CA	N/A	N/A

US-CL-CURRENT: 435/6; 435/325, 435/350, 435/357, 435/363, 435/366, 435/465, 435/7.1, 435/7.72

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

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☐ 13. Document ID: US 5871913 A

L8: Entry 13 of 23 File: USPT Feb 16, 1999

US-PAT-NO: 5871913
DOCUMENT-IDENTIFIER: US 5871913 A

TITLE: DNA encoding the T cell surface protein T4 and use of fragments of T4 in the treatment of AIDS

DATE-ISSUED: February 16, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Maddon; Paul J.	New York	NY	N/A	N/A
Littman; Dan R.	San Francisco	CA	N/A	N/A
Chess; Leonard	Scarsdale	NY	N/A	N/A
Axel; Richard	New York	NY	N/A	N/A
Weiss; Robin	London	N/A	N/A	GB2
McDougal; J. Steven	Atlanta	GA	N/A	N/A

US-CL-CURRENT: 435/6; 536/23.5, 536/24.31

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

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☐ 14. Document ID: US 5869035 A

L8: Entry 14 of 23 File: USPT Feb 9, 1999

US-PAT-NO: 5869035
DOCUMENT-IDENTIFIER: US 5869035 A

TITLE: Methods and compositions for inducing complement destruction of tissue

DATE-ISSUED: February 9, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Link, Jr.; Charles J.	Clive	IA	N/A	N/A
Levy; John P.	West Des Moines	IA	N/A	N/A

US-CL-CURRENT: 424/93.7; 424/277.1, 424/93.21, 435/320.1, 514/44

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw. Desc | Image

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☐ 15. Document ID: US 5830877 A

L8: Entry 15 of 23 File: USPT Nov 3, 1998

US-PAT-NO: 5830877
DOCUMENT-IDENTIFIER: US 5830877 A

TITLE: Method, compositions and devices for administration of naked polynucleotides which encode antigens and immunostimulatory

DATE-ISSUED: November 3, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Carson; Dennis A.	Del Mar	CA	N/A	N/A
Raz; Eyal	San Diego	CA	N/A	N/A

US-CL-CURRENT: 514/44; 536/23.5, 536/23.51, 536/23.52, 536/24.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw. Desc | Image

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☐ 16. Document ID: US 5681746 A

L8: Entry 16 of 23 File: USPT Oct 28, 1997

US-PAT-NO: 5681746
DOCUMENT-IDENTIFIER: US 5681746 A

TITLE: Retroviral delivery of full length factor VIII

DATE-ISSUED: October 28, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bodner; Mordechai	San Diego	CA	N/A	N/A
De Polo; Nicholas J.	Solana Beach	CA	N/A	N/A
Chang; Stephen	Poway	CA	N/A	N/A
Hsu; David Chi-Tang	San Diego	CA	N/A	N/A
Respess; James G.	San Diego	CA	N/A	N/A

US-CL-CURRENT: 435/350; 435/320.1, 435/366, 435/371, 536/23.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

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☐ 17. Document ID: US 5643770 A

L8: Entry 17 of 23 File: USPT Jul 1, 1997

US-PAT-NO: 5643770
DOCUMENT-IDENTIFIER: US 5643770 A

TITLE: Retroviral vector particles expressing complement inhibitor activity

DATE-ISSUED: July 1, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mason; James M.	Wallingford	CT	N/A	N/A
Squinto; Stephen P.	Bethany	CT	N/A	N/A

US-CL-CURRENT: 435/456; 424/93.2, 435/320.1, 435/69.7, 530/350, 536/23.4

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

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☐ 18. Document ID: US 5562904 A

L8: Entry 18 of 23 File: USPT Oct 8, 1996

US-PAT-NO: 5562904
DOCUMENT-IDENTIFIER: US 5562904 A

TITLE: Retroviral transduction of cells using soluble complement inhibitors

DATE-ISSUED: October 8, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Rother; Russell P.	Cheshire	CT	N/A	N/A
Rollins; Scott A.	Monroe	CT	N/A	N/A
Mason; James M.	Wallingford	CT	N/A	N/A
Squinto; Stephen P.	Bethany	CT	N/A	N/A

US-CL-CURRENT: 424/145.1; 424/130.1, 424/141.1, 514/44

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 19. Document ID: US 5422274 A

L8: Entry 19 of 23 File: USPT Jun 6, 1995

US-PAT-NO: 5422274
DOCUMENT-IDENTIFIER: US 5422274 A

TITLE: Internal deletion mutants of soluble T4(CD4)

DATE-ISSUED: June 6, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Maddon; Paul J.	New York	NY	N/A	N/A
Axel; Richard	New York	NY	N/A	N/A
Sweet; Raymond W.	Bala Cynwyd	PA	N/A	N/A
Arthos; James	Ann Arbor	MI	N/A	N/A

US-CL-CURRENT: 435/320.1; 424/188.1, 424/208.1, 435/358, 435/69.4, 435/69.6, 530/388.35, 536/23.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 20. Document ID: US 5126433 A

L8: Entry 20 of 23 File: USPT Jun 30, 1992

US-PAT-NO: 5126433
DOCUMENT-IDENTIFIER: US 5126433 A

TITLE: Soluble forms of the T cell surface protein CD4

DATE-ISSUED: June 30, 1992

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Maddon; Paul J.	New York	NY	N/A	N/A
Chess; Leonard	Scarsdale	NY	N/A	N/A
Axel; Richard	New York	NY	N/A	N/A
Weiss; Robin	London	N/A	N/A	GB2
Littman; Dan R.	San Francisco	CA	N/A	N/A
McDougal; J. Steven	Atlanta	GA	N/A	N/A

US-CL-CURRENT: 530/395; 530/350, 530/380, 530/387.2, 530/387.9, 530/389.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

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☐ 21. Document ID: US 5110906 A

L8: Entry 21 of 23 File: USPT May 5, 1992

US-PAT-NO: 5110906
DOCUMENT-IDENTIFIER: US 5110906 A

TITLE: Derivatives of soluble T-4

DATE-ISSUED: May 5, 1992

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Maddon; Paul J.	New York	NY	N/A	N/A
Axel; Richard	New York	NY	N/A	N/A
Sweet; Raymond W.	Bala Cynwyd	PA	N/A	N/A
Arthos; James	Ann Arbor	MI	N/A	N/A

US-CL-CURRENT: 530/350; 435/5, 435/974, 530/395, 530/821, 930/221

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

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☐ 22. Document ID: AU 9931875 A, WO 9947659 A1

L8: Entry 22 of 23 File: DWPI Oct 11, 1999

DERWENT-ACC-NO: 1999-562109
DERWENT-WEEK: 200008

TITLE: Production of human serum-resistant retroviral vector particles, used particularly for gene therapy, , e.g. for treating inherited and acquired diseases

INVENTOR: MASON, J M

PRIORITY-DATA: 1998US-0040103 (March 17, 1998)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 9931875 A	October 11, 1999	N/A	000	C12N015/00
WO 9947659 A1	September 23, 1999	E	041	C12N015/00

INT-CL (IPC): A01N 63/00; C12N 15/00

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

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☐ 23. Document ID: WO 9604934 A1, EP 769968 A1, EP 769968 A4, JP 10507905 W

L8: Entry 23 of 23 File: DWPI Feb 22, 1996

DERWENT-ACC-NO: 1996-139460
DERWENT-WEEK: 199842

TITLE: Retroviral vectors for use in gene therapy - which are resistant to inactivation by human serum and are produced by cells resistant to lysis by human serum.

INVENTOR: COLLINS, M K L; COSSET, F ; PENSIERO, M D ; TAKEUCHI, Y ; WEISS, R A ; PENSIERO, M

PRIORITY-DATA: 1995US-0451215 (May 26, 1995), 1994US-0291765 (August 17, 1994)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
WO 9604934 A1	February 22, 1996	E	069	A61K048/00
EP 769968 A1	May 2, 1997	E	000	A61K048/00
EP 769968 A4	October 29, 1997	N/A	000	A61K048/00
JP 10507905 W	August 4, 1998	N/A	069	C12N015/09

INT-CL (IPC): A61K 31/70; A61K 35/76; A61K 38/00; A61K 38/21; A61K 38/22; A61K 38/28; A61K 38/46; A61K 39/395; A61K 48/00; C12N 5/00; C12N 5/08; C12N 5/10; C12N 5/22; C12N 7/00; C12N 15/00; C12N 15/09; C12N 15/86

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWMC | Draw. Desc | Image

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L3: Entry 4 of 11 File: USPT Sep 14, 1999

DOCUMENT-IDENTIFIER: US 5952225 A
TITLE: Retroviral vectors produced by producer cell lines resistant to lysis by human serum

ABPL:
Retroviral which are resistant to inactivation by human serum. The retroviral vectors are produced in a cell line which is resistant to lysis by human serum, such cell lines including the HOS, Mv-1-Lu, HT1080, TE671, and human 293 cell lines, as well as cell lines derived therefrom. Such retroviral vectors are especially useful as in vivo gene delivery vehicles.

PCPR:
This invention relates to retroviral vectors which are resistant to inactivation by human serum. More particularly, this application relates to retroviral vectors generated from cells which are resistant to lysis by human serum. In another aspect, this invention relates to gene therapy using such vectors.

DRPR:
FIGS. 1A and 1B are graphs of the time course of virus inactivation by fresh human serum of MLV-A and RD114 viruses generated from NIH3T3 and mink Mv-1-Lu cells;

DRPR:
FIG. 4 shows graphs depicting the sensitivity of MLV-A, MLV-X, and RD114 viruses produced from mink Mv-1-Lu, HOS, and TE671 cell lines to eight different samples of human serum;

DRPR:
FIG. 10 is a graph of titers of retroviral vectors having a VSV-G retroviral envelope wherein said viruses were produced by a gp7C-derived mouse cell line, upon exposure to human serum or inactivated fetal bovine serum;

DRPR:
FIG. 11 is a graph of titers of retroviral vectors produced by the CAK8 cell line, upon exposure to human serum or inactivated fetal bovine serum;

DRPR:
FIG. 12 is a graph of the titers of retroviral vectors produced by the PA317 cell line, upon exposure to human serum or inactivated fetal bovine serum;

DRPR:
FIG. 13 is a graph of the titers of retroviral vectors produced by the CAK8 cell line, upon exposure to human serum for periods of time up to 180 minutes; and

DRPR:
FIG. 14 is a graph of the titers of retroviral vectors produced by the PA317 cell line, upon exposure to human serum for periods of time up to 180 minutes.

DEPR:
Applicants have discovered that when retroviral vectors are produced from a cell line which is resistant to lysis by human serum, such retroviral vectors may be resistant to complement inactivation by human serum, and that such complement resistance is not dependent necessarily upon the envelope employed, even when the envelope is from a murine Type C amphotropic retrovirus.

DEPR:
Thus, the present invention is directed to retroviral vectors which have been produced by a cell line which is resistant to lysis by human serum. The present invention also is directed to gene therapy employing such retroviral vectors, wherein such retroviral vectors contain at least one polynucleotide encoding a therapeutic agent.

DEPR:
In accordance with an aspect of the present invention, there is provided a retroviral vector resistant to inactivation by human serum. The retroviral vector has been produced in a cell line which is resistant to lysis by human serum.

DEPR:
Cell lines which are resistant to lysis by human serum include, but are not limited to, HOS, TE671, HT1080, Mv-1-Lu, and a human 293 cell line, or cell lines dervied from the HOS, TE671, HT1080, Mv-1-Lu, or human 293 cell lines.

DEPR:
The term "cell line derived from the HOS, TE671, HT1080, Mv-1-Lu, or human 293 cell lines," as used herein, means a cell line formed by transfecting one of the above-mentioned cell lines with one or more expression vehicles (e.g., plasmid vectors or retroviral vectors or retroviral vector genomes) including polynucleotides encoding various gag, pol, and env proteins. The gag and pol retroviral proteins may be obtained from any retrovirus, including, but not limited to, Moloney Murine Leukemia Virus, Rous Sarcoma Virus, RD114, BaEV, GALV, SSAV, FeLV-B, human immunodeficiency virus, and avian leukosis virus. Alternatively, the gag/pol proteins may be modified or chimeric gag/pol constructs. The envelope may be an amphotropic envelope, an ecotropic envelope, or a xenotropic envelope, or may be an envelope including amphotropic and ecotropic portions. The envelope may be obtained from any retrovirus, including, but not limited to, Moloney Murine Leukemia Virus, Rous Sarcoma Virus, RD114, BaEV, GALV, SSAV, FeLV-B,

amphotropic murine leukemia viruses (MLV-A), human immunodeficiency virus, avian leukosis virus and NZB virus. Alternatively, the env proteins may be modified or chimeric env constructs, or may be obtained from non-retroviruses, such as vesicular stomatitis virus and HVJ virus. Such cells also may include other polynucleotides such as, for example, polynucleotides encoding selectable markers.

DEPR:
The retroviral vectors, in one embodiment, may be produced by transfecting the cells with a retroviral plasmid vector as described hereinbelow. Alternatively, the retroviral vectors may be produced by infecting the cells with a retroviral vector from another packaging cell. The cells also are provided with the retroviral packaging function. The packaging function may be provided by a replication competent retrovirus, or may be provided by a transient system which includes one or more expression vehicles (e.g., plasmid vectors) including polynucleotides encoding the gag, pol, and env proteins. Such functions also may be provided by transfecting stably the cell line with one or more expression vehicles (e.g., plasmid vectors) including polynucleotides encoding the gag, pol, and env proteins.

DEPR:
As used herein, the term "retroviral plasmid vector" means a plasmid which includes all or part of a retroviral genome including 5' and 3' retroviral long-term repeat (LTR) sequences, a packaging signal (.psi.), and may include one or more polynucleotides encoding a protein(s) or polypeptide(s) of interest, such as a therapeutic agent or a selectable marker. The term "therapeutic" is used in a generic sense and includes treating agents, prophylactic agents, and replacement agents.

DEPR:
In one embodiment, the retroviral plasmid vector may be derived from Moloney Murine Leukemia Virus and is of the LN series of vectors, such as those hereinabove mentioned, and described further in Bender, et al., J. Virol., Vol. 61, pgs. 1639-1649 (1987) and Miller, et al., Biotechniques, Vol. 7, pgs. 980-990 (1989). Such vectors have a portion of the packaging signal derived from a mouse sarcoma virus, and a mutated gag initiation codon. The term "mutated" as used herein means that the gag initiation codon has been deleted or altered such that the gag protein or fragments or truncations thereof, are not expressed.

DEPR:
In another preferred embodiment, the retroviral plasmid vector is derived from Moloney Murine Leukemia Virus and includes at least four cloning, or restriction enzyme recognition sites, wherein at least two of the sites have an average frequency of appearance in eukaryotic genes of less than once in 10,000 base pairs; i.e., the restriction product has an average DNA size of at least 10,000 base pairs. Preferred cloning sites are selected from the group consisting of NotI, SnaBI, SalI, and XhoI. In a most preferred embodiment, the retroviral plasmid

vector includes each of these cloning sites. Such vectors are further described in U.S. Pat. No. 5,672,510, incorporated herein.

DEPR:
When a retroviral plasmid vector including such cloning sites is employed, there may also be provided a shuttle cloning vector which includes at least two cloning sites which are compatible with at least two cloning sites selected from the group consisting of NotI, SnaBI, SalI, and XhoI located on the retroviral plasmid vector. The shuttle cloning vector also includes at least one desired polynucleotide encoding a therapeutic agent which is capable of being transferred from the shuttle cloning vector to the retroviral plasmid vector.

DEPR:
The retroviral plasmid vector includes one or more promoters. Suitable promoters which may be employed include, but are not limited to, the retroviral LTR; the SV40 promoter; and the human cytomegalovirus (CMV) promoter described in Miller, et al., Biotechniques, Vol. 7, No. 9, 980-990 (1989), or any other promoter (e.g., cellular promoters such as eukaryotic cellular promoters including, but not limited to, the histone, pol III, and .beta.-actin promoters). Other viral promoters which may be employed include, but are not limited to, adenovirus promoters, TK promoters, and B19 prvovirus promoters. The selection of a suitable promoter will be apparent to those skilled in the art from the teachings contained herein.

DEPR:
Other retroviral plasmid vectors which may be employed include, but are not limited to, retroviral plasmid vectors derived from Human Immunodeficiency Virus, Rous Sarcoma Virus, avian leukosis virus, NZB virus, the feline endogenous virus RD114, feline leukemia virus B (FeLV-B), simian sarcoma associated virus (SSAV), baboon endogenous virus (BaEV), and gibbon ape leukemia virus (GALV). It is to be understood, however, that the scope of the present invention is not to be limited to any particular retroviral plasmid vector.

DEPR:
Polynucleotides encoding therapeutic agents which may be contained in the retroviral plasmid vector include, but are not limited to, polynucleotides encoding tumor necrosis factor (TNF) genes, such as TNF-.alpha.; genes encoding interferons such as Interferon-.alpha., Interferon-.beta., and Interferon-.gamma.; genes encoding interleukins such as IL-1, IL-.beta.:, and Interleukins 2 through 14; genes encoding GM-CSF; genes encoding adenosine deaminase, or ADA; genes which encode cellular growth factors, such as lymphokines, which are growth factors for lymphocytes; genes encoding epidermal growth factor (EGF), and keratinocyte growth factor (KGF); genes encoding soluble CD4; Factor VIII; Factor IX; cytochrome b; glucocerebrosidase; T-cell receptors; the LDL receptor, ApoE, ApoC, ApoAI and other genes involved in cholesterol transport and metabolism; the alpha-1 antitrypsin (.alpha.1AT) gene; the insulin gene; the hypoxanthine phosphoribosyl transferase gene;

the CFTR gene; negative selective markers or "suicide" genes, such as viral thymidine kinase genes, such as the Herpes Simplex Virus thymidine kinase gene, the cytomegalovirus virus thymidine kinase gene, and the varicella-zoster virus thymidine kinase gene; Fc receptors for antigen-binding domains of antibodies, antisense sequences which inhibit viral replication, such as antisense sequences which inhibit replication of hepatitis B or hepatitis non-A non-B virus; antisense c-myb oligonucleotides; and antioxidants such as, but not limited to, manganese superoxide dismutase (Mn-SOD), catalase, copper-zinc-superoxide dismutase (CuZn-SOD), extracellular superoxide dismutase (EC-SOD), and glutathione reductase; tissue plasminogen activator (tPA); urinary plasminogen activator (urokinase); hirudin; the phenylalanine hydroxylase gene; nitric oxide synthesase; vasoactive peptides; angiogenic peptides; the dopamine gene; the dystrophin gene; the .beta.-globin gene; the .alpha.-globin gene; the HbA gene; protooncogenes such as the ras, src, and bcl genes; tumor-suppressor genes such as p53 and Rb; the LDL receptor; the heregulin-.alpha. protein gene, for treating breast, ovarian, gastric and endometrial cancers; monoclonal antibodies specific to epitopes contained within the .beta.-chain of a T-cell antigen receptor; the multidrug resistance (MDR) gene; polynucleotides encoding ribozymes; antisense polynucleotides; genes encoding secretory peptides which act as competitive inhibitors of angiotensin converting enzyme, of vascular smooth muscle calcium channels, or of adrenergic receptors, and polynucleotides encoding enzymes which break down amyloid plaques within the central nervous system. It is to be understood, however, that the scope of the present invention is not to be limited to any particular therapeutic agent.

DEPR:
In another embodiment, the retroviral vector is produced by introducing a wild-type retrovirus into a cell line which is resistant to lysis by human serum, and recovering the resistant retroviral vector from the cell line. In one embodiment, the retrovirus is selected from the group consisting of Rous Sarcoma Virus, RD114, BaEV, SSAV, FeLV-B, GALV, avian leukosis virus, and murine leukemia viruses, such as, for example, Moloney Murine Leukemia Virus or amphotropic murine leukemia viruses (MLV-A), including, but not limited to, strains 4070A and 1504.

DEPR:
Preferably, the cell line is selected from the group consisting of HOS, TE671, HT1080, Mv-1-Lu, human 293 cells, or cell lines derived therefrom.

DEPR:
Applicants have found that when retroviral vectors are produced by introducing the above-mentioned retroviruses into the above-mentioned cell lines, the resulting retroviral vectors produced by the above-mentioned retrovirus/cell line combinations are resistant to inactivation by human complement proteins which are found in human serum.

DEPR:
Alternatively, a retroviral vector may be produced by introducing into a pre-packaging cell (i.e., a cell including polynucleotides encoding gag and pol proteins), which is resistant to lysis by human serum, a plasmid vector including a polynucleotide which encodes an envelope protein, such as a retroviral env protein, and a retroviral plasmid vector including a 5' LTR and a 3' LTR, a packaging signal, and at least one polynucleotide encoding a protein or polypeptide of interest. The gag and pol retroviral proteins may be obtained from any retrovirus, including, but not limited to, Moloney Murine Leukemia Virus, Rous Sarcoma Virus, the feline endogenous virus RD114, BaEV, gibbon ape leukemia virus (GALV), simian sarcoma associated virus SSAV, FeLV-B, human immunodeficiency virus, NZB virus, and avian leukosis virus. Alternatively, the gag/pol proteins may be modified or chimeric gag/pol constructs. The envelope may be obtained from any retrovirus, including, but not limited to, Moloney Murine Leukemia Virus, amphotropic murine leukemia viruses (MLV-A), Rous Sarcoma Virus, the feline endogenous virus RD114, BaEV, gibbon ape leukemia virus (GALV), SSAV, FeLV-B, human immunodeficiency virus, NZB virus, and avian leukosis virus. The envelope also may be an envelope which includes amphotropic and ecotropic portions. Alternatively, the env proteins may be modified or chimeric env constructs or obtained from non-retroviruses, such as vesicular stomatitis virus and HVJ virus.

DEPR:
Thus, in accordance with another aspect of the present invention, there is provided a retroviral vector resistant to inactivation by human serum which has been produced by introducing into a pre-packaging cell line, which is resistant to lysis by human serum, a plasmid vector including a polynucleotide encoding an envelope protein which may be obtained from a retrovirus including those selected from the group consisting of Moloney Murine Leukemia Virus, Rous Sarcoma Virus, the feline endogenous virus RD114, BaEV, SSAV, FeLV-B, gibbon ape leukemia virus (GALV), amphotropic murine leukemia viruses (MLV-A), human immunodeficiency virus, NZB virus, avian leukosis virus; or an envelope which includes amphotropic and ecotropic portions; or may be obtained from a non-retrovirus, such as vesicular stomatitis virus or HVJ virus; or which may be a modified or chimeric env construct; and a retroviral plasmid vector including a 5' LTR, a 3' LTR, a packaging signal, and at least one polynucleotide encoding a protein or polypeptide of interest. In accordance with yet another aspect of the present invention, there is provided a packaging cell line for generating retroviral vectors resistant to inactivation by human serum which includes a polynucleotide encoding an envelope protein as hereinabove described. Preferably, the packaging cell line includes a first plasmid vector including a polynucleotide encoding the gag and pol retroviral proteins, and a second plasmid vector encoding an envelope protein as hereinabove described. The packaging cell line is resistant to lysis by human serum.

DEPR:
As stated hereinabove, the polynucleotide encoding the gag and pol retroviral proteins may be obtained from any retrovirus, including, but not limited to, Moloney Murine Leukemia Virus, amphotropic murine leukemia viruses (MLV-A), Rous Sarcoma Virus, the feline endogenous virus RD114, BaEV, gibbon ape leukemia virus (GALV), SSAV, FeLV-B, NZB virus, and avian leukosis virus (ALV). Alternatively, the gag/pol proteins may be modified or chimeric gag/pol constructs. In general, the polynucleotide encoding the gag and pol retroviral proteins is contained in an appropriate plasmid vector. In one embodiment, the gag and pol retroviral proteins are obtained from Moloney Murine Leukemia Virus, and are contained in a plasmid known as pCRIPenv-, as described in Danos, et al., Proc. Nat. Acad. Sci., Vol. 85, pgs. 6460-6464 (1988).

DEPR:
Another plasmid vector includes a polynucleotide encoding an envelope protein which may be obtained from any retrovirus, including, but not limited to, Moloney Murine Leukemia Virus, Rous Sarcoma Virus, the feline endogenous virus RD114, BaEV, SSAV, FeLV-B, gibbon ape leukemia virus (GALV), amphotropic murine leukemia viruses (MLV-A), human immunodeficiency virus, NZB virus, and avian leukosis virus, or non-retroviruses, such as vesicular stomatitis virus and HVJ virus, or modified or chimeric env constructs. In one alternative, the envelope protein includes amphotropic and ecotropic portions. The plasmid vectors encoding the gag and pol proteins, and the env protein and the retroviral plasmid vector hereinabove described, then are transfected into a cell which is resistant to lysis by human serum to provide a helper-free packaging cell line which will generate retroviral particles resistant to inactivation by human serum and which include gag and pol proteins such as, for example, those hereinabove described, an envelope protein which may be obtained from any retrovirus, including, but not limited to, Moloney Murine Leukemia Virus, Rous Sarcoma Virus, the feline endogenous virus RD114, BaEV, SSAV, FeLV-B, gibbon ape leukemia virus (GALV), amphotropic murine leukemia viruses (MLV-A), human immunodeficiency virus, NZB virus, avian leukosis virus, or non-retroviruses, such vesicular stomatitis virus and HVJ virus, or a modified or chimeric env construct, or an envelope including amphotropic and ecotropic portions. Such cell line may further include a retroviral plasmid vector including at least one polynucleotide encoding a protein or polypeptide of interest, such as a therapeutic agent, which may include those hereinabove described. Thus, the packaging cell line becomes a producer cell line which generates a retroviral vector which is resistant to inactivation by human serum which also includes at least one polynucleotide encoding a therapeutic agent. Such retroviral vector particles may be employed in gene therapy procedures such as those herein described, and may be administered to a human host in dosages such as those herein described.

DEPR:
In a preferred embodiment, the retroviral vector is produced in

a cell line which is a human 293 cell line or a cell line derived from a human 293 cell line.

DEPR:
Applicants have discovered surprisingly that, when retroviral vectors are produced from a human 293 cell line or a cell line derived from a human 293 cell line, that such retroviral vectors are resistant to complement inactivation by human serum, and that such complement resistance is not dependent upon the envelope employed, even when the envelope is an amphotropic envelope.

DEPR:
The retroviral vectors, which are administered in vivo to an animal, can be produced by providing the human 293 cell line with the retroviral packaging function. Such packaging function may be provided by a replication competent retrovirus, or may be provided by a transient system which includes one or more expression vehicles (e.g., plasmid vectors) including polynucleotides encoding the gag, pol, and env proteins. Such functions also may be provided by stably transfecting the 293 cell line with one or more expression vehicles (e.g., plasmid vectors) including polynucleotides encoding the gag, pol, and env proteins. In addition to providing the 293 cells with the polynucleotides encoding the gag, pol, and env proteins, the 293 cells also are transfected with a retroviral plasmid vector as described hereinabove.

DEPR:
The retroviral plasmid vector is transfected into the packaging cell line which is a human 293 cell line or a cell line which is derived from a human 293 cell line, whereby such packaging cell line becomes a producer cell line that generates retroviral vectors which are resistant to inactivation by human serum. The packaging cell line derived from human 293 cells may be generated, for example, by immortalizing the cell line by transformation with transforming proteins such as the simian virus 40 (SV40) large tumor antigen; alternatively, one may use immortalized 293 cell lines such as 293T or 293E. Once the appropriate 293 cell line is chosen, it is necessary to co-transfect these cells with a plasmid encoding retroviral gag/pol proteins such as, for example, Moloney Murine Leukemia Virus gag/pol proteins, along with a plasmid containing a selectable marker such as hygromycin. Individual hygromycin resistance clones are isolated and screened both for the presence of the gag proteins (p15, p12, p30, and p10) and for reverse transcriptase (RT) activity. Routinely the clone expressing the highest levels of p30 and RT is designated the pre-packaging cell line. To generate a packaging cell line, it is necessary to co-transfect the pre-packaging cell line with an env-containing plasmid and a plasmid containing a selectable marker different than the one used in the first step (e.g., puromycin resistance, etc.). If the env component is toxic to the cell, such as is the case with many fusogenic envelopes, then it may be necessary to express this from an inducible promoter. Clones then are screened, and an appropriate packaging cell line is chosen. Such packaging cell lines also

may be prepared as disclosed in Pear, et al., September 1993, or in PCT Application No. W094/19478, published Sep. 1, 1994. In one embodiment, the packaging cell line is the CAK8 (or Bing) cell line (ATCC No. CCRL 11554), which is an amphotropic envelope-expressing packaging line, whereby the resultant retrovirus generated by such cell line has an amphotropic envelope. In another embodiment, the cell line is the Bosc 23 (ATCC No. CCRL 11270), which is an ecotropic envelope-expressing packaging line, whereby the resultant retrovirus generated by such cell line has an ecotropic envelope.

DEPR:
Thus, in accordance with another aspect of the present invention, there is provided a complement-resistant retroviral vector, which may be produced from a cell line selected from the group consisting of the HOS, TE671, HT1080, Mv-1-Lu, and human 293 cell lines, or a cell line derived from the HOS, TE671, HT1080, Mv-1-Lu, and human 293 cell lines, which includes an envelope selected from the group consisting of VSV-G envelope protein; Moloney Murine Leukemia Virus envelope; Rous Sarcoma Virus envelope; feline endogenous virus RD114envelope; gibbon ape leukemia virus envelope; baboon endogenous virus envelope; simian sarcoma associated virus envelope; amphotropic murine leukemia virus (MLV-A) envelope; human immunodeficiency virus envelope; avian leukosis virus envelope; NZB viral envelopes; and HVJ virus envelope.

DEPR:
The retroviral vectors are administered to an animal in vivo in an amount effective to produce a therapeutic effect in the animal. The animal may be a mammal, including human and non-human primates. The retroviral vectors may be administered systemically, for example, intravenously or intraarterially or intraperitoneally. The vectors also may be administered subcutaneously or intramuscularly. The retroviral vectors, which are resistant to inactivation by human serum, transduce cells in vivo, whereby the transduced cells express the therapeutic agent in vivo.

DEPR:
The retroviral vectors are administered to an animal in an amount effective to produce a therapeutic effect in the animal. In general, the retroviral vectors are administered in an amount of at least 10.sup.5 cfu, and in general such amount does not exceed 10.sup.12 cfu. Preferably, the retroviral vectors are administered in an amount of from about 10.sup.6 cfu to about 10.sup.10 cfu. The exact dosage to be administered is dependent upon various factors, including the age, height, weight, and sex of the patient, the disorder being treated, and the severity thereof.

DEPR:
The retroviral vectors are administered to the patient in a pharmaceutically acceptable carrier, such as, for example, a physiological saline solution. Other pharmaceutical carriers include, but are not limited to, mineral oil, alum, and lipid

vesicles such as liposomes. The selection of a suitable pharmaceutical carrier is deemed to be within the scope of those skilled in the art from the teachings contained herein.

DEPR:
The retroviral vectors are useful in the treatment of a variety of diseases including but not limited to adenosine deaminase deficiency, sickle cell anemia, thalassemia, hemophilia, diabetes, .alpha.-antitrypsin deficiency, brain disorders such as Alzheimer's disease, and other illnesses such as growth disorders and heart diseases, for example, those caused by alterations in the way cholesterol is metabolized and defects of the immune system.

DEPR:
In one embodiment, the retroviral vectors may include a negative selectable marker, such as, for example, a viral thymidine kinase gene, and more particularly, the Herpes Simplex Virus thymidine kinase (TK) gene. Such retroviral vectors may be administered to tumor cells (in particular to cancer cells) in a human patient in vivo. The retroviral vectors then transduce the tumor cells. After the retroviral vectors have transduced the tumor cells, the patient is given an interaction agent, such as gancyclovir or acyclovir, which interacts with the protein expressed by the negative selectable marker in order to kill all replicating cells (i.e., the tumor cells) which were transduced with the retroviral vector including the negative selectable marker.

DEPR:
The retroviral vectors mentioned hereinabove also may be administered in an animal model for determining the effectiveness of a gene therapy treatment. For example, a retroviral vector, produced in a cell line which is resistant to lysis by human serum, and including a polynucleotide encoding a therapeutic agent, may be administered to animals of the same species in varying amounts. From determining the effectiveness of the gene therapy treatment in the animal, one may determine an effective amount of the retroviral vector to be administered to a human patient.

DEPR:
In another embodiment, the cells which are resistant to lysis by human serum, which have been transfected with a retroviral plasmid vector such as hereinabove described, which includes one or more polynucleotides encoding a therapeutic agent, whereby such cells have become producer cells, are administered to a patient in vivo, whereby the producer cells generate in vivo retroviral vector particles including a polynucleotide encoding a therapeutic agent.

DEPR:
Such an embodiment is applicable particularly to the treatment of tumors (including malignant and non-malignant tumors) such as, for example, brain tumors and head and neck tumors. For example, the producer cells may include a retroviral plasmid vector including a negative selectable marker. The producer

cells then are administered to the tumor, whereby the producer cells generate retroviral vector particles including the polynucleotide encoding the negative selectable marker. The retroviral vector particles generated by the producer cells transduce the tumor cells, whereby the tumor cells produce the negative selectable marker. Upon administration of an interaction agent to the patient, the transduced tumor cells are killed.

DEPR:
Alternatively, the retroviral vector may transduce eukaryotic cells, in vitro, whereby the eukaryotic cells are cultured in vitro for the in vitro production of the therapeutic agent, or, alternatively, the transduced eukaryotic cells may be administered to a host as part of a gene therapy procedure, whereby the transduced eukaryotic cells express the therapeutic agent in vivo in a host.

DEPR:
In accordance with another aspect of the present invention, there is provided a method of identifying retroviral vectors that are resistant to inactivation by human serum comprising introducing a retrovirus into a cell line in which a resultant retroviral vector is to be produced. The resistance of the resultant retroviral vector to inactivation by human serum then is determined. The determination of the resistance of the resultant retroviral vector to inactivation by human serum may be made by methods described hereinbelow in the examples.

DEPR:
In accordance with another aspect of the present invention, there is provided a method of producing retroviral vectors resistant to inactivation by human serum. The method comprises determining resistance of cells to lysis by human serum. Resistance of cells to lysis by human serum may be determined by methods such as those described hereinbelow. Retroviral vectors then are produced from those cells found to be resistant to lysis by human serum.

DEPR:
Murine NIH 3T3 cells (ATCC No. CRL1658) were cultivated in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% newborn calf serum. Mink MV-1-Lu cells (ATCC No. CCL6584) were cultivated in DMEM with 10% fetal calf serum.

DEPR:
The MFGnlslacZ genome (Ferry, et al., Proc. Nat. Acad. Sci., Vol. 88, pgs. 8377-8381 (1991) was introduced into NIH 3T3 and Mv-1-Lu cells by infection with lac Z (contained in Murine Leukemia Virus-A) produced from the .psi.CRIP packaging line containing MFGnlslacZ genome, as described in Tailor, et al., J. Virol., Vol. 67, pgs. 6737-6741 (1993). MFGnlslacZ is a Moloney Murine Leukemia Virus based retroviral vector including a 5' LTR, a 3' LTR, a packaging signal, and a lacZ gene. After cell cloning by limiting dilution, clones which gave high titers of lac Z pseudotype in a pilot rescue experiment were selected. Lac Z pseudotypes containing helper virus were

produced by infection of these cell clones with replication competent MLV-A 1504 strain. (Rasheed, et al., J.Virol., Vol. 19, pgs. 13-18 (1976); Sommerfelt, et al., Virology, Vol. 176, pgs. 58-69 (1990); Tailor, et al., 1993). Viruses were harvested in serum-free Opti-MEM (Gibco U.K.). On the day before harvest, cells were washed once with Opti-MEM and incubated in Opti-MEM at 37.degree. C. for 1 hour. The medium then was replaced with fresh Opti-MEM and cells were incubated overnight. Culture supernatant was harvested, filtered through a 0.45 .mu.m filter, aliquoted, and stored as virus stock at -70.degree. C. until use. All virus stocks had original lac Z titers ranging from 2.times.10.sup.4 to 4.times.10.sup.6 on appropriate assay cells.

DEPR:
40 .mu.l of virus stock was mixed with an equal volume of fresh human serum or heat-inactivated human serum in 20 mM HEPES buffer, pH 7, and incubated at 37.degree. C. for up to 1 hour. After incubation, the virus-serum mixture was diluted serially from 1:13 to 1:1,300 with DMEM/10% FCS with 8 .mu.g/ml Polybrene and plated on TE671 cells in 24 well plates. Assay cells were seeded at 5.times.10.sup.4 cells/well in 24 well plates on the day before infection. After 4 hours of infection, virus was removed and cells were cultivated in growth medium. Two days after infection, the cells were stained with X-gal in situ and lac Z positive colonies were counted as described. (Tailor, et al., 1993). Relative titers (%) for fresh (F) and heat-inactivated (HI) serum treatment are shown in FIG. 1A. As shown, in FIG. 1A, lac Z (MLV-A) from NIH 3T3 cells is indicated as 3T3A, and lac Z (MLV-A) from Mv-1-Lu cells is indicated as Mink A.

DEPR:
As shown in FIG. 1A, the virus produced from murine NIH 3T3 cells was inactivated rapidly. Five minutes of exposure to human serum decreased the viral titer more than 3 logs. The virus produced from Mv-1-Lu cells also was inactivated. After 1 hour of exposure to human serum, the viral titer was decreased by 2 logs.

DEPR:
In another experiment, murine NIH 3T3 cells and mink Mv-1-Lu cells were cultured as hereinabove described.

DEPR:
The MFGnlslacZ genome was introduced into NIH 3T3 and Mv-1-Lu cells, and then clones which gave high titers of lacZ pseudotype were selected as hereinabove described.

DEPR:
Lac Z (RD114) containing helper virus then was obtained from a NIH 3T3 cell clone with MFGnlslacZ genome by transfection with full length proviral DNA (sc3c, provided by Dr. S. O'Brien and described further in Reeves, et al., J. Virol., Vol. 52, pgs 164-171 (1984)). A cell clone which gave high titers of lac Z pseudotype was selected. Lac Z pseudotypes containing helper virus were produced by infection of an Mv-1-Lu cell clone

containing MFGnlslacZ genome with a replication competent RD114virus as described previously. (Tailor, et al., 1993). The viruses were harvested in Opti-MEM, filtered through a 0.45 .mu.m filter, and frozen at -70.degree. C. until use. All virus stocks had original lac Z titers ranging from 2.times.10.sup.4 to 4.times.10.sup.6 on appropriate assay cells.

DEPR:
Virus stocks were mixed with equal volumes of fresh human serum or heat-inactivated human serum as hereinabove described, and incubated as hereinabove described. The virus-serum mixtures then were plated on TE671 cells as hereinabove described, virus then was removed, and the cells were cultivated in growth medium as mentioned hereinabove. The cells then were stained with X-gal in situ and lacZ positive colonies were counted as hereinabove described. Relative titers (%) for fresh (F) and heat-inactivated (HI) serum treatment are shown in FIG. 1B. As shown in FIG. 1B, lacZ (RD114) obtained from NIH3T3 cells is indicated as 3T3RD, and lacZ (RD114) obtained from mink Mv-1-Lu cells is indicated as Mink RD.

DEPR:
As shown in FIG. 1B, the titer of lacZ (RD114) produced from NIH3T3 cells was decreased by 2 logs within 5 minutes when exposed to normal human serum, whereas lacZ (RD114) produced by Mv-1-Lu cells was resistant to a 1 hour exposure to human serum. As shown in FIGS. 1A and 1B, the activity in human serum responsible for viral inactivation was heat labile. The results shown in FIGS. 1A and 1B show that viruses produced by Mv-1-Lu cells are more resistant to human serum than those produced by NIH3T3 cells.

DEPR:
Murine NIH3T3 and PG13 (Miller, et al., J. Virol., Vol. 65, pgs. 2220-2224 (1991) ATCC No. 10686), GP+EAM12 (Markowitz, et al., Virology, Vol. 167, pgs. 400-406 (1988)), and GP+E86 (Markowitz, et al., J. Virol., Vol. 82, pgs. 1120-1124 (1988)) packaging cell lines were cultivated in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% newborn calf serum. Mink Mv-1-Lu, dog Cf2ThS+L- (ATCC No. CRL1430), human HOS (ATCC No. CRL1543), and human TE671 cells (Sommerfelt, et al., 1990; Tailor, et al., 1993, ATCC No. CRL8805) were cultivated in DMEM supplemented with 10% fetal calf serum.

DEPR:
The MFGnlslacZ genome was introduced into NIH3T3, Mv-1-Lu, Cf2ThS+L-, HOS, and TE671 cells by infection with lacZ (contained in MLV-A) produced from the .psi.CRIP packaging line (Danos, et al., 1988) as described in Tailor, et al., 1993. After cell cloning by limiting dilution, clones which have high titer of lacZ pseudotype in a pilot rescue experiment were selected. LacZ pseudotypes containing helper virus were produced by infection of these cell clones with replication competent MLV-A 1504 strain, MLV-XNZB, RD114, BaEVM7, SSAV, GALV-SF, and FeLV-B as described in Tailor, et al., 1993. Viruses were harvested in either serum-free Opti-MEM or DMEM/10% fetal calf serum, filtered through a 0.45.mu.m filter,

and frozen at -70.degree. C. until use. LacZ (RD114) was obtained from NIH3T3 cells by transfection with full-length proviral DNA (sc3c, provided by Dr. S. O'Brien, and described further in Reeves, et al., J. Virol., Vol. 52, pgs. 164-171 (1984)). Helper-free pseudotypes from NIH3T3 cells were obtained from PG13, GP+EAM12, and GP+E86 packaging lines either by transduction with helper-free lacZ (contained in MLV-A) or transfection of MFGnlslacZ. Helper-free pseudotypes from Mv-1-Lu cells were obtained by transfection of Mv-1-Lu cells containing MFGnlslacZ with separate expression plasmids encoding MLV gag/pol genes and MLV-E, MLV-A, or RD114envelope genes. All virus stocks had original lacZ titers ranging from 2.times.10.sup.4 to 4.times.10.sup.6 on appropriate assay cells.

DEPR:
40.mu.l of virus dilution was mixed with an equal volume of fresh human serum, or heat-inactivated human serum in 20 mM HEPES buffer pH 7, with less than 2% fetal calf serum from virus harvest and incubated at 37.degree. C. for up to 1 hour. After incubation, virus-serum mixture was diluted with 1 ml DMEM with 8 .mu.g/ml Polybrene and plated on the assay cells in 24 well plates. MLV-E was assayed on NIH 3T3 cells; MLV-A, MLV-X (Levy, Science, Vol. 182, pgs. 1151-1153 (1973)), BaEV (Benveniste, et al., Nature, Vol. 248, pgs. 17-20 (1974)), and RD114(McAllister, et al., Nature New Biol., Vol. 235, pgs. 3-6 (1972)) were assayed on Mv-1-Lu or TE671 cells, and GALV (Kawakami, et al., Nature New Biol., Vol. 235, pgs. 170171 (1972)), SSAV (Thielen, etal., J. Natl. Cancer Inst., Vol 47, pgs. 881-889 (1971)), and FeLV-B (Jarrett, et al., J. Gen. Virol., Vol. 20, pgs. 169-175 (1973)) were assayed on TE671 cells. Assay cells were seeded at 5.times.10.sup.4 cells/well in 24 well plates on the day before infection. After 4 hours of infection, virus was removed and cells were cultivated in growth medium. Two days after infection, the cells were stained with X-gal in situ and lacZ positive colonies were counted as described in Tailor, et al., 1993. Relative titers (%) for fresh and heat-inactivated human serum treatment versus fetal calf serum (FCS) treatment are shown in FIGS. 2A, 2B, 2C, 2D, and 2E. FIG. 2A shows virus titers for viruses generated from murine NIH3T3 cells, PG13 cells, GP+E86 cells, or GP+Am12 cells. FIG. 2B shows virus titers for viruses generated from dog Cf2ThS+L- cells; FIG. 2C shows virus titers for viruses generated from mink Mv-1-Lu cells; FIG. 2D shows virus titers for viruses generated from human HOS cells, and FIG. 2E shows virus titers for viruses generated from human TE671 cells.

DEPR:
As shown in FIG. 2A, retroviruses produced from NIH3T3 cells, as well as from GP+E86, PG13, and GP+Am12 cells were sensitive to inactivation by fresh human serum. In addition, viruses produced by the dog cell line Cf2ThS+L- all were sensitive to inactivation by human serum (FIG. 2B). As shown in FIG. 2C, however, while two murine leukemia viruses (MLV-A and MLV-X) produced by Mv-1-Lu cells were sensitive to human serum, the RD114, BaEV, and GALV viruses were resistant to inactivation by human serum. MLV-X was found to be sensitive to inactivation by

human serum when produced by HOS or TE671 cells (FIGS. 2D and 2E). MLV-A was found to be resistant to complement inactivation when produced by HOS cells. RD114and BaEV were found to be resistant when produced by these cell lines. GALV and FeLV-B were partially resistant when produced by these cell lines, and SSAV, which is closely related to GALV (Delassus, et al., Virology, Vol. 173, pgs. 205:213 (1989)), was resistant.

DEPR:
In order to determine which RD114viral gene product(s) conferred resistance to human serum, recombinant virions were produced from Mv-1-Lu cells by expressing MLV gag and pol genes in combination with either MLV or RD114env genes, as hereinabove described. The helper-free pseudotypes were obtained by transfection of MFGnlslacZ/Mv-1-Lu cells with separate expression plasmids encoding MLV gag and pol genes, and MLV-A, MLV-E, or RD114env genes. The gag/pol plasmid was pCRIP env-, described in Danos, et al., 1988. Plasmids expressing the MLV-A, MLV-E, or RD114env genes were derived from FB3 described in Heard, et al., J. Virol., Vol. 65, pgs. 4026-4032 (1990) by inserting the MLV-A, MLV-E, or RD114env genes downstream of the FB29 LTR promoter. The MLV-A virus, strain 4070A, indicated in Table I below, is described in Danos, et al., 1988. As shown in Table I below, virions with RD114envelope were resistant to treatment with three fresh human serum samples for 1 hour, whereas those with amphotropic or ecotropic MLV envelopes were more sensitive. This demonstrates that envelope sequences can control sensitivity, in agreement with the assignment of p15E as the viral protein which triggers complement activation. (Bartholomew, et al., 1978.)

DEPR:
NIH3T3, Mv-1-Lu, Cf2ThS+L-, HOS, and TE671 cells containing MFGnlslacZ provirus, were infected with MLV-A, MLV-X, or RD114virus, as described in Examples 1 and 2. 2.times.10.sup.6 uninfected or infected cells then were removed from the plates with EDTA, washed, and resuspended in 200 .mu.l of sodium [.sup.51 Cr] chromate (1 mCi/ml, Amersham) at 37.degree. C. for 1 hour. After labeling, the cells were washed and resuspended in DMEM with 10% FCS. After incubation at 37.degree. C. for 30 minutes, the cells were collected by centrifugation, washed with serum-free DMEM, and resuspended in serum-free DMEM at 2.times.10.sup.5 cells/ml. 50.mu.l of cell suspension was mixed with 100.mu.l of serum dilution in a V-bottom microtiter well. Serum dilutions were 2/3, 2/9, 2/27, and 2/81. The plates were incubated at 37.degree. C. for 1 hour and the percent-specific .sup.51 Cr released into cell-free supernatant was determined by the following formula:

DEPR:
The results of this assay are shown in FIG. 3. As shown in FIG. 3, NIH3T3 cells and Cf2ThS+L- cells were highly sensitive to lysis by human serum. Mv-1-Lu, HOS, and TE671 cells were resistant to lysis. Viral infection of the cells did not affect their sensitivity. Infection of Mv-1-Lu or HOS cells with MLV-A or MLV-X did not render them sensitive to lysis by human serum.

These data suggest that a cellular factor(s), which can protect the uninfected cells from lysis by human serum, may be incorporated into virions produced from mink and human cells. Alternatively, a cellular factor(s), which enhances cell lysis by human serum may be incorporated into virions produced from mouse and dog cells. This latter possibility was supported by an observation that retroviruses made by mouse and dog cells were found to be inactivated by human serum mainly via recognition of sugar epitopes, Gal (.alpha.1-3) Gal epitopes, by natural antibodies. (Takeuchi, et al., unpublished data.) A viral effect, which does not affect the lysis of infected producer cells, also controls the differential sensitivity of viruses produced from a given cell.

DEPR:
MLV-A, MLV-X, and RD114viruses produced from mink Mv-1-Lu cells, HOS cells, or TE671 cells, as described in Examples 1 and 2, were treated with eight samples of normal human serum (also hereinafter referred to as NHS-1 through NHS-8) at 37.degree. C. for 1 hour, and then the viruses were plated on TE671cells. Relative titers (%) versus FCS treatment, of surviving lacZ psuedotypes are shown together with the mean value of relative titers in FIG. 4.

DEPR:
As shown in FIG. 4, RD114produced from Mv-1-Lu or HOS cells was universally resistant. Results obtained with TE671cells showed a more variable pattern of inactivation. The above results indicate that resistance to inactivation may not only be dependent upon the species of animal from which the producer cell line is obtained, but also may vary between cell lines from a particular species.

DEPR:
MLV-A and RD114viruses were produced from NIH3T3 cells or mink Mv-1-Lu cells as described in Examples 1 and 2. The viruses were harvested in serum-free Opti-MEM.

DEPR:
In another experiment, MLV-A and RD-114 viruses generated from NIH3T3 or mink Mv-1-Lu cells were tested for lysis by serum samples NHS-3, NHS-7, and C7D-2 in a reverse transcriptase assay. In this assay, 12 ml of cell supernatant was harvested from confluent producer cells in serum-free Opti-MEM and clarified by low speed centrifugation and by filtration at 0.45 .mu.m. The virus was concentrated by ultracentrifugation (12,000.times.g, 1 hour, 4.degree. C.). The viral pellet then was suspended in 120 .mu.l of cold Opti-MEM and aliquoted in 4 tubes (30 .mu.l each). 30 .mu.l of 0.5% Triton, FCS, and human serum were added, and the tubes were incubated at 1 hour at 37.degree. C. Reverse transcriptase activity was measured as described in Goff, et al., J. Virol., Vol. 38, pgs. 239-248 (1981). 80 .mu.l of reverse transcriptase mix containing 5 .mu.l of Tris (1M, pH 8.0), 5 .mu.l dithiothreitol (0.1M), 2.5 .mu.l MnCl.sub.2 (0.04M), 10 .mu.L KCl (1M), 1 .mu.l primer-template (1 mg/ml of poly (rA), p(dT) 12-18, Pharmacia), 31.5 .mu.l H.sub.2 O, and 20 .mu.l .sup.3 H-TTP (0.1 mCi/ml of

[Me-.sup.3 H]-thymidine triphosphate, Amersham), and 20 .mu.l of virus/serum mixture were added to wells of a 96 well plate. Duplicate reactions were incubated at 37.degree. C. Polymerized TTP was separated from free TTP on DE81 filter mats pre-wetted with 2.times.SSC using a cell harvester. Filters were washed for 30 seconds with 2.times.SSC, dried, and each spot was counted. Percent specific reverse transcriptase released by serum was estimated according to the formula:

DEPR:
The results shown in Table 2 indicate that depletion of complement from normal human serum, either by addition of cobra venom factor or complement component C1, resulted in the loss of its ability to inactivate MLV-A produced from NIH3T3 cells or Mv-1-Lu cells, and RD114produced from NIH3T3 cells. Thus, as reported previously (Welsh, et al., 1975), the inactivation of MLV-A and RD114by human sera was due to complement. C1addition specifically depletes the classical pathway of complement activation. The above data, therefore, is in agreement with the previous report of involvement of the classical pathway of retroviral inactivation by human serum. (Cooper, et al., J. Exp. Med., Vol. 144, pgs. 970-984 (1976)).

DEPR:
In addition, as reported previously (Welsh, et al., 1975), normal human serum was able to cause reverse transcriptase release from virions (Table 3). The results shown in Tables 2 and 3 also showed that MLV-A produced from Mv-1-Lu cells was more sensitive to lysis than RD114, and virus produced from NIH3T3 cells was more sensitive than virus produced from Mv-1-Lu cells. The C7 deficient serum which inhibited viral infectivity, however, failed to induce virion lysis. The above data demonstrate that some steps in activation of complement by the classical pathway, prior to the final stage of virion lysis, is sufficient for inhibition of retroviral infection by human serum.

DEPR:
Plasmid pG1Na was derived from plasmid pG1. Plasmid pG1was constructed from pLNSX (Palmer, et al., Blood, Vol. 73, pgs. 438-445). The construction strategy for plasmid pG1is shown in FIG. 5. The 1.6 kb EcoRI fragment, containing the 5' Moloney Murine Sarcoma Virus (MoMuSV) LTR, and the 3.0 kb EcoRI/ClaI fragment, containing the 3' LTR, the bacterial origin of replication and the ampicillin resistance gene, were isolated separately. A linker containing seven unique cloning sites was then used to close the EcoRI/ClaI fragment on itself, thus generating the plasmid pGO. The plasmid pGO was used to generate the vector plasmid pG1(FIG. 7) by the insertion of the 1.6 kB EcoRI fragment containing the 5' LTR into the unique EcoRI site of pGO. Thus, pG1(FIG. 7) consists of a retroviral vector backbone composed of a 5' portion derived from MoMuSV, a short portion of qaq in which the authentic ATG start codon has been mutated to TAG (Bender, et al. 1987), a 54 base pair multiple cloning site (MCS) containing, from 5' to 3', the sites EcoRI, NotI, SnaBI, SalI, BamHI, XhoI, HindIII, ApaI, and ClaI and a 3' portion of MoMuLV from base pairs 7764 to 7813

(numbered as described (Van Beveren, et al., Cold Spring Harbor, Vol. 2, pg. 567, 1985) (FIG. 6). The MCS was designed to generate a maximum number of unique insertion sites, based on a screen of non-cutting restriction enzymes of the pG1plasmid, the neo.sup.r gene, the .beta.-galactosidase gene, the hygromycin.sup.r gene, and the SV40 promoter.

DEPR:
This example compares the sensitivity of retroviral vectors including pG1Na containing either an amphotropic envelope produced from PA317 cells, an amphotropic envelope from human CAK8 cells, or a VSV-G envelope from a stable mouse cell line (gp7).

DEPR:
The CAK8 cell line (ATCC No. CCRL 11554) was derived from the 293 T cell line (Pear, et al., Proc. Nat. Acad. Sci., Vol. 90, pgs. 8392-8396 (September 1993). The CAK8 cell line includes a polynucleotide encoding a retroviral envelope derived from the amphotropic 4070A retrovirus, wherein the polynucleotide sequence encoding the 35 C-terminal amino acids of 4070A retrovirus is replaced with a polynucleotide encoding the 35 C-terminal amino acids of an ecotropic virus. A clone, termed 293T/17, was isolated from a 293T population (Du Bridge, et al., Mol. Cell. Biol., Vol. 7, pgs. 379-387 (1987)) that produced retroviral supernatants capable of infecting NIH 3T3 cells at titers greater than 10.sup.6 /ml following transient transfection with wild-type Moloney virus (pZap) (Shoemaker, et al., J. Virol., Vol. 40, pgs. 164-172 (1981)), and a .beta.-galactosidase-expressing retroviral vector pBND8 (Pear, et al., 1993). The gag-pol expressing plasmid, pCripEnv-(Danos, et al., Proc. Nat. Acad. Sci., Vol. 85, pgs. 6460-6464 (1988)), which contains a mutation in the envelope region, lacks the packaging site, and replaces the 3' LTR with the SV40 poly (A) site, was transfected into 293T/17 cells along with a plasmid conferring hygromycin resistance. (Bernard, et al., Exp. Cell. Res., Vol. 158, pgs. 237-243 (1985)). Individual clones were selected and tested for reverse transcriptase activity (Goff, et al., J. Virol., Vol. 38, pgs. 239-248 (1981)), and one clone, Anjou 65, had the highest reverse transcriptase activity. The amphotropic envelope expressing construct, pCripAMgag-(Danos, et al., 1988), which contains mutations in the gag region, lacks the packaging site, and replaces the 3' LTR, was transfected into Anjou 65 cells along with a plasmid expressing the gpt resistance gene. (Jasin, et al., Genes and Dev., Vol. 2, pgs. 1353-1363 (1988)). Individual clones were isolated and tested for the ability to produce high titer .beta.-galactosidase-expressing retroviruses. One clone produced .beta.-gal retrovirus with a titer in excess of 10.sup.6 /ml following transfection with pBND8. Two rounds of limiting dilution subcloning were performed subsequently, giving rise to the CAK8, or Bing cell line.

DEPR:
Amphotropic G1Na retroviral supernatant was generated by transient transfection of the CAK8 packaging cell line or of PA317 cells as described in Pear, et al., 1993. Retroviral

supernatant was harvested 48 hours post-transfection. VSV-G pseudotyped G1Na retroviral vector (provided by Dr. Elio Vanin, St. Jude Children's Research Hospital, Memphis, Tennessee) was prepared from a stable mouse cell line (gp7) containing a VSV-G envelope under a tetracycline inducible promoter and an ecotropic gag/pol.

DEPR:
A retroviral vector genome containing the neo.sup.R gene (G1Na) was introduced into GP7C cells by exposure to culture medium from a producer clone (G1Na.40, Genetic Therapy, Inc., Gaithersburg, Md.) generating amphotropic vector particles. (G1Na.40was generated by transducing the PA317 cell line with pG1Na). Individual clones were isolated by G418selection and culture media from each was assayed for content of vector RNA by RNA slot blot analysis. A clone designated GP7CN having the highest apparent titer, was selected.

DEPR:
As shown in FIG. 10, when the VSV-G pseudotyped G1Na retroviral vector was incubated with human serum for 30 minutes, a 35-fold decrease in titer was observed. No titer loss was seen with heat-inactivated fetal bovine serum, and a minimal loss of titer (approximately 2-fold) was observed with heat-inactivated human serum.

DEPR:
In contrast to the results obtained with the VSV-G pseudotyped retroviral vector, amphotropic G1Na vector produced from the CAK8 cell line was completely stable in the presence of human serum (FIG. 11), whereas the G1Na vector produced from PA317 cells resulted in a 50-fold loss in titer after incubation with human serum for 30 minutes (FIG. 12).

DETL:
TABLE I Inactivation of Recombinant Viruses Produced from Mv-1-Lu Cells Relative Titer (%) Virus Serum Sample 1 Serum Sample 2 Serum Sample 3 MLV-A (1502) 2.7 5.5 10 RD114 120 116 75 MLV gag/pol + MLV-A 21 21 18 (4070A) env MLV gag/pol + 104 94 71 RD114 env MLV gag/pol + 32 23 26 MLV-E env

CLPR:
1. A packaging cell for producing retroviral vectors resistant to inactivation by human serum, said packaging cell comprising a cell resistant to lysis by human serum, said packaging cell containing a polynucleotide encoding a portion of a virus selected from the group consisting of the feline endogenous virus RD114, BaEV, SSAV, FeLY-B, NZB virus, avian leukosis virus, and HVJ virus, said portion of said virus comprising at least the envelope protein, wherein said packaging cell does not include the entire viral RNA of the feline endogenous virus RD114, BeEV,SSAV, FeLV-B, NZB virus, avian leukosis virus, or HVJ virus.

CLPR:

10. The cell of claim 1 wherein said cell is selected from the group consisting of the HOS, TE671, HT1080, MV-1-Lu, and human 293 cell lines, and cell lines derived from the HOS, TE671, HT1080, Mv-1-Lu, and human 293 cell lines.

CLPR:
12. A producer cell for producing retroviral vectors resistant to inactivation by human serum, said producer cell being resistant to lysis by human serum and including: (i)a polynucleotide encoding a retroviral envelope proteins, said polynucleotide encoding said envelope protein being obtained from a virus selected from the group consisting of feline endogenous virus RD114, BaEV,SSAV, FeLV-B, NZB virus, avian leukosis virus, and HVJ virus, and (ii) a retroviral vector including a 5' LTR, a 3' LTR, a packaging signal, and at least one polynucleotide encoding a protein or polypeptide of interest, wherein said producer cell does not include the entire viral RNA of feline endogenous virus RD114, BaEV,SSAV, FeLV-B, NZB virus, avian leukosis virus, or HVS virus.

CLPR:
20. A process for producing retroviral vector particles resistant to inactivation by human serum, comprising; generating retroviral vector particles from the producer cell of claim 12, said retroviral vector particles being resistant to inactivation by human serum.

CLPR:
21. A method of producing retroviral vectors resistant to inactivation by human serum, comprising:

CLPV:
producing retroviral vectors from cells determined to be resistant to lysis by human serum.

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